

I claim:

1. A method of preparing a transplantable graft of an engineered tissue equivalent for treatment of body tissue defects, comprising:

5 a) preparing an assemblable culture insert for producing a transplantable graft with living cells; said assemblable culture insert comprising:

 i) a female slip slave vessel having holes in its bottom wall to create a pathway for cell culture medium exchange between outside and inside a lower chamber of said vessel when the insert is placed in a cell culture container;

10 ii) a male slip ring vessel insertable in said female slip slave vessel with a supporting membrane to create an upper inner chamber; and

 iii) a sheet of an extraembryonic biological supporting membrane that creates said upper inner chamber and lower chamber when mounted and stretched between the intact inserted assembly of the slip slave vessel and the male slip ring vessel;

15 b) preparing seed cells of main functional cells and stromal cells;

 c) constructing a tissue equivalent graft of a histological configuration of mammalian tissue through seeding and growing living cells on said extraembryonic biological supporting membrane; and

20 d) disassembling said culture insert and releasing the tissue equivalent graft for treatment of body tissue defects.

2. The method of claim 1 wherein said tissue equivalent graft includes any one of:

 a) skin equivalent;

 b) mucosal equivalent;

25 c) corneal equivalent; and

 d) other body membrane equivalents.

3. The method of claim 1 wherein said tissue equivalent graft is employed for any one of:

- a) repairing skin defects;
 - b) reconstructing mucosal surfaces for treatment of mucosal defects;
 - c) constructing ocular surface for treatment of ocular surface defects;
 - d) healing of corneal epithelial defects; and
 - e) repairing any body membrane defects.
- 5 4. The method of claim 1 wherein said [a] transplantable graft is an engineered skin substitute for treatment of skin defects.
- 10 5. The method of claim 4 wherein said engineered skin substitute is an equivalent structure of skin having a surface of epidermal cell layer and an underline dermal layer including connective cells and tissue matrix.
- 15 6. The method of claim 4 wherein said engineered skin substitute is one of:
 - a) autologous graft comprising said biological supporting membrane and autologous epidermal and dermal cells;
 - b) semi-autologous graft comprising said biological supporting membrane and autologous epidermal cells and allogenic dermal cells;
 - c) allogenic graft comprising said biological supporting membrane and allogenic epidermal and dermal cells;
 - d) fibroblast graft comprising said biological supporting membrane and fibroblasts;
- 20 7. The method of claim 5 wherein said epidermal cell are living keratinocyte derived from epidermis of skin.
- 25 8. The method of claim 5 wherein said epidermal cell are living keratinocyte derived from hair follicle.
9. The method of claim 5 wherein said connective cells are living fibroblasts derived from dermis of skin.
10. The method of claim 5 wherein said tissue matrix is composed of connective fibers.
11. The method of claim 4 wherein said skin defects are skin ulcers caused by different chronic diseases.

12. The method of claim 4 wherein said skin defects are skin wounds caused by different skin injuries.
13. The method of claim 4 wherein said skin defects are caused by surgery.
14. The method of claim 1 wherein said extraembryonic biological supporting membrane is a de-epithelial amnion, and has a layer of basement membrane and layers of connective tissue.
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15. The method of claim 1 wherein said extraembryonic biological supporting membrane is a de-traphoblast chorion, and has a layer of pseudo-basement membrane and layers of connective tissue.
- 10 16. The method of claim 1 wherein said cell culture container is a cell culture dish or a cell culture plate
17. The method of claim 1 wherein said construction of the graft comprises:
 - a) assembling the insert and placing the insert in said cell culture container;
 - b) removing the epithelium and exposing the basement membrane of amnion;
 - 15 c) seeding the living cells on the basement membrane side and connective tissue side through chambers of the insert;
 - d) growing the cells on the membrane with culture medium; and
 - e) disassembling the insert and releasing the transplantable graft.
18. The method of claim 1 wherein construction of the graft comprises:
 - c) assembling the insert and placing the insert in said cell culture container;
 - d) removing the traphoblast layer and exposing the pseudo-basement membrane of chorion;
 - 20 e) seeding the living cells on the basement membrane side and connective tissue side through chambers of the insert;
 - d) growing the cells on the membrane with culture medium; and
 - e) disassembling the insert and releasing the transplantable graft.
19. The method of claim 1 wherein growing living cells is enhanced on/in a biological

supporting membrane, by:

- a) forming an acidic solution of collagen;
- b) adding the solution to a chamber of the insert and raising the solution to a pH level sufficient to precipitate collagen onto said extraembryonic biological supporting membrane;
- c) incubating the solution with the membrane in the insert before seeding cells; and
- d) forming a cell culture medium with multiple factors.

20. A method of inhibiting cell differentiation of keratinocytes, comprising:

- a) forming a keratinocyte culture medium with a low concentration of calcium; and
- b) culturing the keratinocytes with the medium.